consistent with a study in normals in which increasing doses of norepinephrine produced increased randomness of heart rate patterns (2). This hypothesis could easily be tested using multiple methods. One would be to plot the HRV power spectrum. If there were, indeed, an increase in vagal modulation of heart rate, there would be a clear increase in the size of a clearly seen peak in the high-frequency band. If an increase in randomness occurred, any increase in high-frequency power would be associated with an increasingly broad and abnormal-looking peak.

Alternatively, the Poincaré plot (a plot of each normal-to-normal interval vs. the next) could be generated. Increased randomness would be associated with an increasingly complex-looking plot (3). Finally, nonlinear HRV indices could be calculated. The calculation of the short-term fractal scaling exponent (4) would normally require about 1,000 beat-segments of data. The ratio of the axes of an ellipse fitted to the Poincaré plot (SD12) could be calculated for the same 5-min segments that were used for HRV. Increasing values of SD12, or decreasing values of the short-term scaling exponent, would be consistent with increasing randomness, rather than increased levels of autonomic modulation of the heart. Indeed, this technique has previously been applied to ventricular tachycardias, and increased SD12 was found to precede arrhythmic events (5).

Phyllis K. Stein, PhD
Washington University School of Medicine
HRV Laboratory
4625 Lindell Boulevard, Suite 402
St. Louis, MO 63108
E-mail: pstein@im.wustl.edu

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REPLY

We thank Dr. Stein for her interest and comments. Although we would like to study nonlinear methods in our patients, this task was prohibitive in the time frame required for this response. We appreciate the suggestions and will explore our data using these methods in the future.

Dr. Stein suggests that postoperative patients behave similarly to healthy volunteers in whom increased randomness of the heart-period signal was attributed to increasing doses of norepinephrine (1) and that the increases in both time- and frequency-domain parameters of heart rate variability (HRV) observed in our study do not represent vagal resurgence (2). We disagree with this hypothesis for several reasons.

First, in a study of patients undergoing major thoracic or abdominal surgery, we showed persistent downregulation and desensitization of the lymphocyte beta-adrenergic receptor/adenylyl cyclase system, which correlated with decrements in time- and frequency-domain indices of HRV throughout the first week after surgery (3). These changes occurred in the absence of change in perioperative epinephrine or norepinephrine levels.

Second, to suggest that our atrial fibrillation (AF) patients (n = 48) had a significantly different perioperative neurohumoral response than did that of controls (n = 48) matched for age, gender, and identical operation, appears unlikely. Finally, the HRV response seen in our control group was very similar to that seen in other patients undergoing major thoracic surgery, supporting the presence of parasympathetic withdrawal and not resurgence (4).

David Amar, MD
Memorial Sloan-Kettering Cancer Center
Anesthesiology
1275 York Ave.
Rm M-304
New York, NY 10021
E-mail: amard@mskcc.org

Alan H. Kadish, MD

doi:10.1016/j.jacc.2004.05.010

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Measurement of Circulating Vascular Endothelial Growth Factor in Obese Subjects

I read with interest the report by Rehman et al. (1) evaluating the circulating levels of hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) in obese subjects. Although the investigators should be congratulated by their results on the study of HGF, I would like to underline some methodological concerns regarding the measurement of VEGF levels.

Serum VEGF is not a suitable indicator of circulating extracellular VEGF levels at the time of sampling; VEGF is stored in the α-granules of platelets and is released during blood clotting. As a consequence, VEGF level in the serum is several-fold higher than that in matched plasma samples (2,3). In plasma, platelet degranulation is minimized by adding anticoagulants to the blood samples; in particular, CTAD (citrate, theophylline, adenosine, dipyridamole) plasma is recommended for the measurement of

circulating extracellular VEGF (4). The investigators (1) stated that "serum was separated after coagulation," but they did not report the interval between venipuncture and separation of serum from blood cell; this interval should be standardized and declared. In serum, VEGF concentrations increase in a time-dependent manner (5). In a clinical situation, where blood samples are taken and left for variable times before processing, the contribution from the clotting process would make the measurement unreliable (5). Allowing the whole blood sample to clot for between 2 and 6 h before serum is collected reduces time-dependent, nonuniform release of VEGF (6). In addition, the researchers did not report the conditions of processing (centrifugal force and the length of centrifugation), which are relevant and should be standardized.

Finally, a direct correlation between platelet counts and serum VEGF has been described (6). When serum is used for the measurement of VEGF, it is advisable to correct the results for variations in platelet count and platelet size (3).

In conclusion, meticulous standardization of sampling is a mandatory step in studies on blood VEGF levels. The investigators' conclusion that "serum VEGF levels were not significantly elevated in obese versus lean subjects" cannot be justified on the basis of the data presented.

Simone Ferrero, MD
Department of Obstetrics and Gynecology
San Martino Hospital
University of Genoa
Largo R. Benzi 1
16132 Genoa
Italy
E-mail: simone.ferrero@fastwebnet.it

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REPLY

We would like to thank Dr. Ferrero for the comments regarding our study, which demonstrated that obesity was associated with an increase in serum hepatocyte growth factor (HGF) levels (1). Our study also showed a nonsignificant trend toward higher vascular endothelial growth factor (VEGF) levels in the serum of obese subjects. As Dr. Ferrero correctly points out, the time between collection of blood samples and the time when the serum is centrifuged can affect the concentrations of growth factors like VEGF. We are not aware of any clear directional bias in our study related to serum collection that would have resulted in low levels of serum VEGF in obese subjects and thus masked higher circulating VEGF levels. However, VEGF levels did demonstrate marked interindividual variability, and this may have contributed to the absence of a statistically significant difference of VEGF levels between obese and nonobese subjects. It is quite possible that serum HGF levels may be more robustly associated with obesity, whereas serum VEGF levels may be a reflection of not just obesity but also other co-morbidities and factors like the serum clotting time.

Furthermore, a more recent study was indeed able to show that serum VEGF levels are associated with obesity (2). Interestingly, the researchers in that study found that visceral fat area showed a better correlation with circulating VEGF levels than did body mass index. This may reflect a greater contribution of visceral fat to circulating VEGF levels than other fat tissues.

Finally, we believe that the association between obesity and vascular growth factor is an exciting and rapidly growing area of research, and it will continue to enhance our knowledge of how obesity can affect both the vasculature and atherosclerosis.

Jalees Rehman, MD
Robert V. Considine, PhD
Keith L. March, MD, PhD, FACC
Indiana Center for Vascular Biology and Medicine
975 W. Walnut St. IB 441
Indianapolis, IN 46202
E-mail: kmarch@iupui.edu

doi:10.1016/j.jacc.2004.05.015

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